Plectin Gene Mutations Can Cause Epidermolysis Bullosa with Pyloric Atresia

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Epidermolysis bullosa with pyloric atresia (EB-PA), manifesting with neonatal blistering and gastric anomalies, is known to be caused by mutations in the hemidesmosomal genes *ITGA6* and *ITGB4*, which encode the α6 and β4 integrin polypeptides, respectively. As part of our molecular diagnostics program, we have now encountered four families with EB-PA in which no mutations could be identified in these two genes. Instead, PCR amplification followed by heteroduplex scanning and/or direct nucleotide sequencing revealed homozygous mutations in the plectin gene (*PLEC1*), encoding another hemidesmosomal protein previously linked to EB with muscular dystrophy. Our findings provide evidence for additional molecular heterogeneity in EB, and emphasize the importance of screening EB-PA patients not only for α6β4 integrin but also for plectin deficiency.

Key words: Molecular diagnostics/blistering disorders/genodermatoses/hemidesmosomal proteins
J Invest Dermatol 124:111–115, 2005

Epidermolysis bullosa (EB) is a phenotypically diverse group of heritable mechanobullous disorders characterized by blistering and erosions of the skin and mucous membranes (Fine *et al*, 2000). Ten different genes expressed within the cutaneous basement membrane zone are now known to harbor mutations that underlie different forms of EB (Pulkkinen and Uitto, 1999; Uitto and Richard, 2004). Adding to the phenotypic complexity of EB is the fact that several well-characterized variants are associated with extracutaneous manifestations with considerable morbidity and mortality (Uitto *et al*, 1997; Fine *et al*, 2000). One of these variants, EB with pyloric atresia (EB-PA; OMIM #226730), manifests with neonatal blistering associated with PA, a combination that can lead to early postnatal demise of the affected individuals. EB-PA has been shown to result in most families from mutations in the genes encoding the subunit polypeptides of α6β4 integrin, *ITGA6* and *ITGB4*, respectively (Pulkkinen and Uitto, 1998; Pulkkinen *et al*, 1998). Another variant, EB with muscular dystrophy (EB-MD; OMIM #226670), is characterized by neonatal blistering accompanied by proximal muscle weakness that can develop during childhood (early onset) or in the third or fourth decade of life (late onset). EB-MD is caused by mutations in the gene encoding plectin, *PLEC1* (GeneBank U53204), which is expressed not only in the hemidesmosomes but also in the sarcolemma and the Z-lines of the skeletal muscle (Uitto *et al*, 1996).

As part of the diagnostic services to the global EB patient community provided by the DebRA Molecular Diagnostics Laboratory, which was established at Jefferson Medical College in 1996, we have analyzed approximately 1000 families with different forms of EB, including 35 families with EB-PA. A total of 56 distinct mutations in the EB-PA families have been identified in the *ITGB4* gene (see Pulkkinen *et al*, 1998; Nakano *et al*, 2001) and four of them in the *ITGA6* gene (Pulkkinen *et al*, 1997; Ruzzi *et al*, 1997). In this report, we describe four cases with EB and PA and neonatal lethality in which analysis of the *ITGB4* and *ITGA6* genes, including direct sequencing of exons and flanking intronic sequences, yielded no pathogenetic mutations. Subsequent mutation analysis of *PLEC1*, however, identified homozygous mutations in each case.

The proband in each family was a newborn with clinical findings of blistering and PA, and they died from complications of the disease shortly after birth. Information on the families as well as clinical and diagnostic features of the proband are included in Fig 1 and Table I. These studies were approved by the Institutional Review Board of Thomas Jefferson University, and they adhere to Declaration of Helsinki principles. A written informed consent was obtained from the patients or their guardians. PCR amplification of 33 exons of *PLEC1*, followed by heteroduplex scanning and/or direct dideoxynucleotide sequencing of the probands' and/or parents' DNA resulted in identification of homozygous mutations in each family (Fig 2). The parents were found to be heterozygous carriers of the corresponding mutations, consistent with consanguinity in each family (see Fig 1). Two of the mutations, Q305X and Q3029X (cases 2 and 3, respectively), were nonsense mutations resulting from C-to-T transitions and reflecting hypermutability of putative 5-methylcytosine within exons 10 and 33, respectively. One of the mutations (case 1) was an out-of-frame deletion, 1563del4, predicting a premature stop-codon 30 bp downstream from the site of deletion within exon 15. Finally,

Abbreviations: EB, epidermolysis bullosa; EB-MD, EB with muscular dystrophy; EB-PA, EB with pyloric atresia

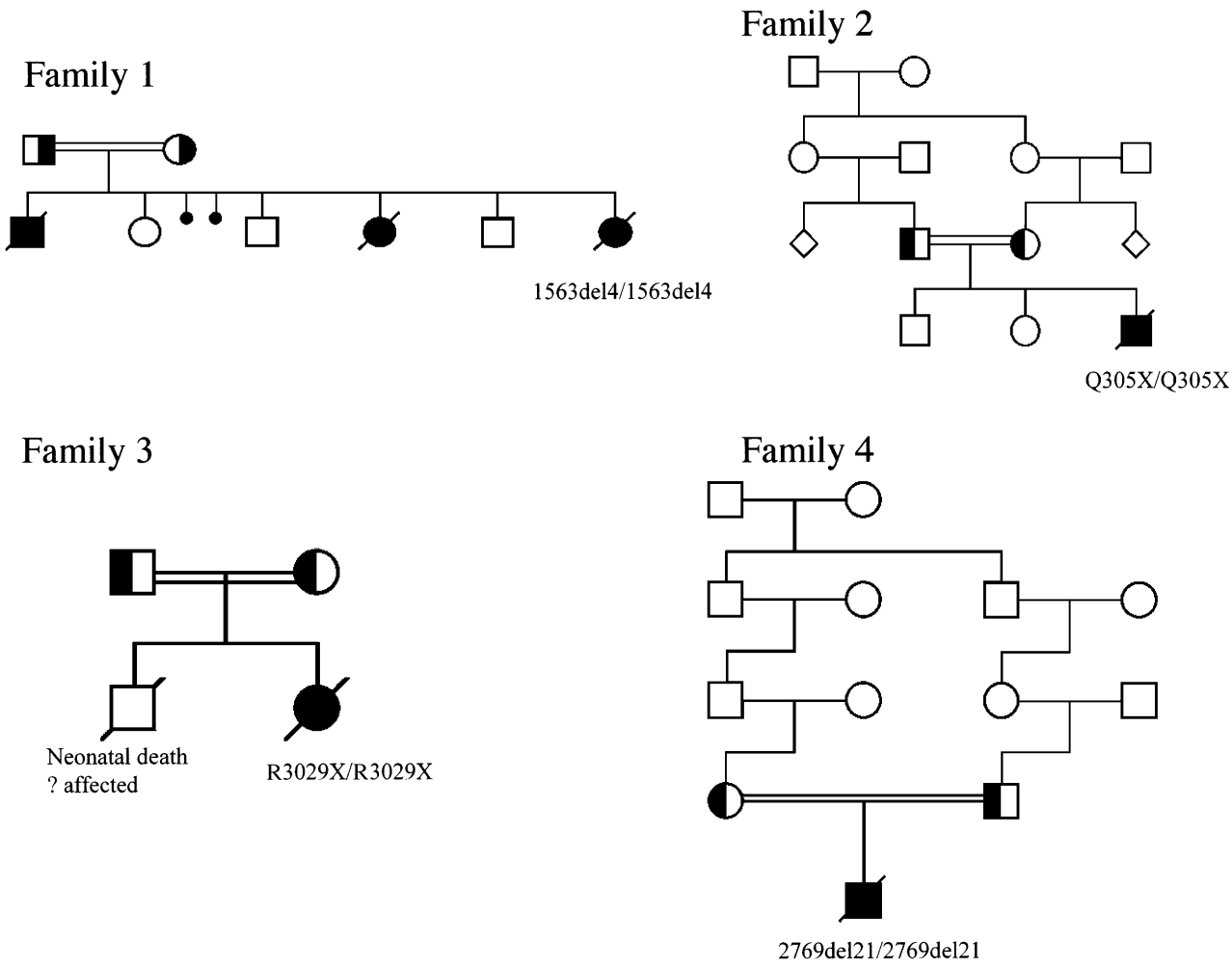

Figure 1
Nuclear pedigrees of the families with epidermolysis bullosa with pyloric atresia (EB-PA). Solid symbols denote children who died from complications of the disease shortly after birth. Note consanguinity in each family.

Table I. Plectin gene mutations in families with epidermolysis bullosa with pyloric atresia (EB-PA)

Family no.	Ethnic origin	Consanguinity (parents)	Clinical features of the proband	Diagnostic skin[a] pathology	Mutations[b] maternal/paternal
1	Pakistani	Distant cousins	Blistering and PA at birth; two similarly affected older siblings	EM: low basal cell cytolysis; attenuation of anchoring filaments; rudimentary hemidesmosomes	1563del4/1563del4 (1614del4/1614del4)
2	Lebanese	First cousins	Extensive blistering; aplasia cutis of abdomen and legs; ear abnormalities	IF: laminin 5, uncein, and type VII collagen expressed at the base of an intra-epidermal cleft	Q305X/Q305X
3	Saudi Arabian	First cousins	Blistering and PA at birth; another sibling with neonatal demise	IF: $\alpha 6\beta 4$ staining normal	R3029X/R3029X
4	Caucasian	Second cousins	Extensive blistering and aplasia cutis at birth; poly-hydramnion	EM: lamina lucida cleavage; hypoplastic hemidesmosomes; IF: collagen XVII/BPAG2 staining negative; collagen VII staining normal	2769del21/2769del21 (2820del21/2829del21)

[a]EM, electron microscopy; IF, immunofluorescence; Please note that immunofluorescence for plectin was not done in any of the cases.
[b]The mutations in the plectin gene (*PLEC1*; GeneBank U53204) refer to nucleotide positions of the gene counting the translation initiation codon ATG as 1–3, as published by McLean *et al* (1996); the numbers in parentheses refer to positions of the corresponding nucleotides counting the beginning of the published gene sequence (−51) as 1.

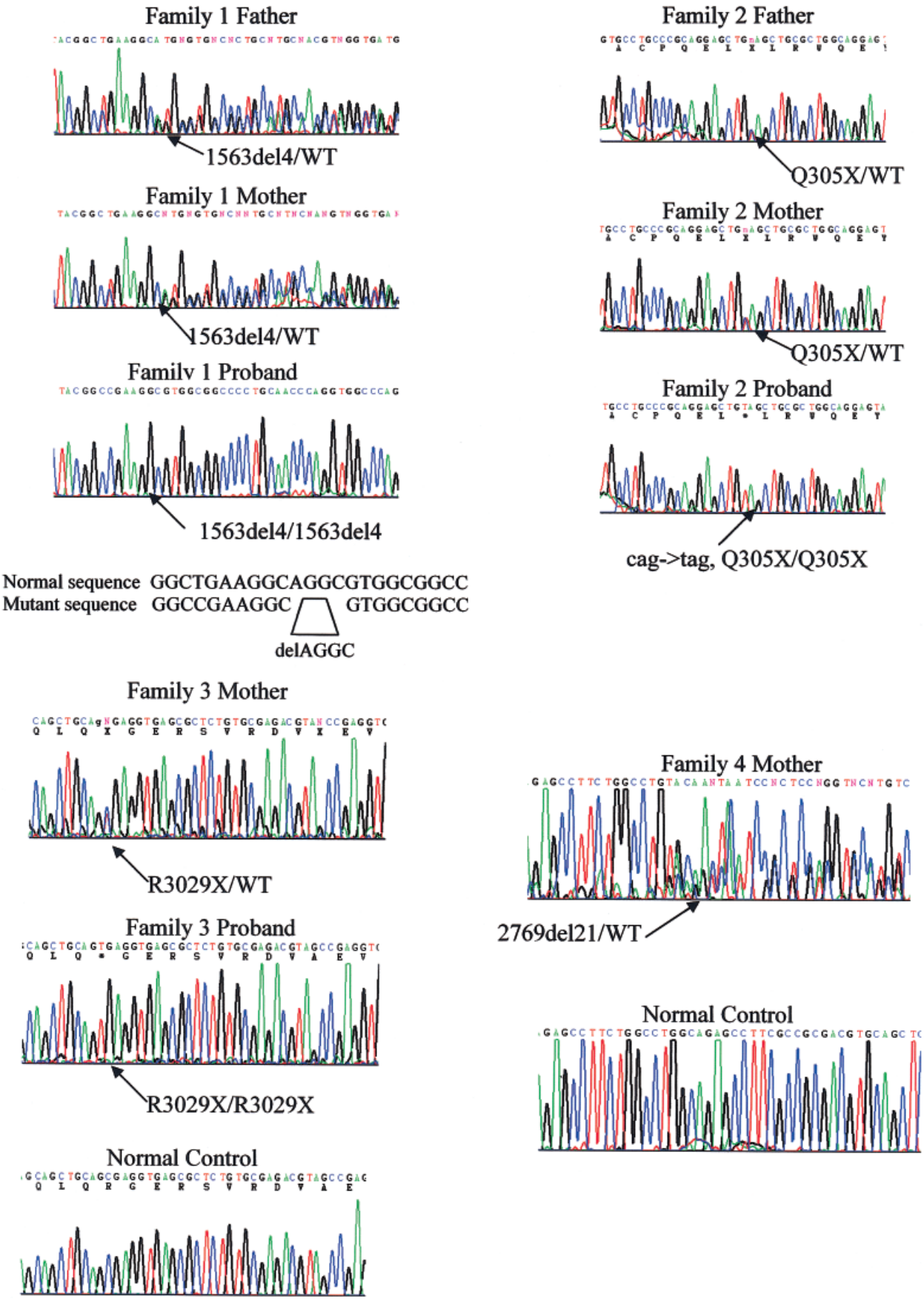

Figure 2
Nucleotide sequencing data documenting mutations in the *PLEC1* gene in Families 1–4 with epidermolysis bullosa (EB) with pyloric atresia. Family 1: The proband is homozygous and the parents are heterozygous for a 4-bp deletion mutation, 1563del4. Family 2: The proband is homozygous and the parents are heterozygous for the nonsense mutation Q305X. Family 3: The proband is homozygous and the parents (mother only shown) are heterozygous for the nonsense mutation R3029X. Family 4: The parents (mother only shown) are heterozygous for a 21-bp deletion mutation. No DNA was available from the proband, whereas a normal control sequence is shown for reference.

another mutation (case 4) was a 21-bp in-frame deletion, 2769del21, within exon 23, resulting in loss of seven amino acids.

Plectin, a large ($\sim$ 500 kDa) multidomain adhesion molecule, is expressed in a broad spectrum of tissues and cells, including epithelial cells, muscle, and neural tissues (Wiche, 1998). In the skin, plectin is concentrated at the basal layer of epidermal cells where it is a component of both hemidesmosomes and desmosomes (Koster *et al*, 2004). In hemidesmosomes, this protein directly links the intermediate filaments to the cytoplasmic domain of the β4 integrin subunit and to the 180 kDa bullous pemphigoid antigen, a transmembrane collagen, type XVII (Schaapveld *et al*, 1998; Koster *et al*, 2003). The corresponding gene, *PLEC1*, is located on chromosomal locus 8q24 in the human genome and encodes 33 exons (Liu *et al*, 1996; McLean *et al*, 1996). Analysis of the murine plectin gene has revealed that there are as many as 16 plectin variants because of alternatively spliced exons in the 5′ region of the gene, and the tissue expression of the various plectin variants is different, suggesting distinct functions for the different isoforms (Elliott *et al*, 1997; Fuchs *et al*, 1999).

Plectin mutations have previously been identified in EB-MD, an autosomal recessive disorder with neonatal blistering and delayed, progressive MD (McLean *et al*, 1996; Pulkkinen *et al*, 1996; Smith *et al*, 1996; Rouan *et al*, 2000). These mutations are, in general, nonsense mutations or out-of-frame insertions or deletions within exon 32, and they result in premature stop-codons predicting truncated polypeptides and downregulation of the corresponding mRNA through nonsense-mediated mRNA decay (Shimizu *et al*, 1999). In these patients, immunofluorescence with a monoclonal antibody (Mab 121), which is directed against an epitope within the plectin rod domain (Okumura *et al*, 1999), is often completely negative. Several cell types, including keratinocytes, express a naturally occurring splice variant of plectin that lacks the rod domain (Elliott *et al*, 1997), and it is conceivable, therefore, that low levels of the truncated polypeptides are expressed from the mutant alleles. Nevertheless, the pathogenetic role of plectin mutations in these patients is attested to by mice in which the plectin gene has been inactivated by targeted ablation. These mice, in addition to blistering within the basal keratinocytes, show necrotic muscle fibers with disorganized myofibrils and sarcomeres (Andra *et al*, 1997). In addition to autosomal recessive EB-MD, an autosomal dominant form of EB simplex, the Ogna variant, has been shown to result from a heterozygous missense mutation (R2110W) (Koss-Harnes *et al*, 2003). These patients do not develop muscle symptoms, and there is no evidence of PA.

This report provides a description of plectin mutations in multiple families with EB-PA, and similar observations have been made on two Japanese patients with EB-PA and neonatal lethality (Nakamura *et al*, 2004). Furthermore, a recent study (Charlesworth *et al*, 2003) reported a novel homozygous plectin mutation (2727del14) associated with a lethal form of recessive EB in a consanguinous family with three affected offspring. This new variant of EB was characterized by generalized blistering, aplasia cutis of the limbs, complex developmental anomalies, and rapid demise after birth, clinical features shared by the patients in our series. One of the three siblings was suspected by ultrasound at the 25th wk of gestation to have "obstruction of the gastric outlet", but the occurrence of PA was subsequently not substantiated (Charlesworth *et al*, 2003).

The majority of patients with EB-PA have mutations in the α6β4 integrin genes. It should be noted, however, that a homozygous missense mutation (G931D) in the cytoplasmic tail of β4 integrin has been reported in a form of non-Herlitz junctional EB without PA (Inoue *et al*, 2000). Nevertheless, the gastrointestinal phenotype, i.e., PA, reported in these patients may be related to perturbed interactions between plectin and α6β4 integrin within attachment structures expressed during gastrointestinal development (Rezniczek *et al*, 1998; Nievers *et al*, 2000; Koster *et al*, 2001). In particular, the in-frame deletion 2769del21 in case 4 results in elimination of seven amino acids that may be critical for such interactions. It is also of interest that two of the stop-codon causing mutations reported in this study reside within exons 15 (case 1) and 10 (case 2), upstream from the previously reported stop-codon mutations within the rod domain. In these cases, differences in the size of the truncated polypeptides, if expressed at low levels, may explain the phenotypic variability (EB-PA *vs* EB-MD) resulting from mutations in the *PLEC1* gene. Finally, the stop-codon mutation in exon 33 (case 3) could have led to muscle involvement, similar to other premature-termination codon mutations in exon 32; however, this hypothesis could not be verified because of early demise of the affected individual.

Collectively, our findings attest to additional molecular heterogeneity in EB, and they emphasize the importance of screening of EB-PA patients by immunofluorescence not only for α6β4 integrin but also for plectin deficiency. Identification of mutations in the plectin gene provides the opportunity for prenatal diagnosis in these families at risk for recurrence of EB-PA (Pfendner *et al*, 2003).

The authors thank Carol Kelly for assistance. Drs Jo-David Fine, Nauriya Al-Sannaa, Peter Mamunes, Ivan Lo, and Sean McCandless assisted in providing clinical information, Dr Arnoud Sonnenberg provided helpful discussion, and Drs. Hiroshi Shimizu and Daisuke Sawamura shared unpublished information. These studies were supported by The National Institutes of Health and The Dystrophic Epidermolysis Bullosa Research Association of America.

DOI: 10.1111/j.0022-202X.2004.23564.x

Manuscript received July 16, 2004; revised September 10, 2004; accepted for publication September 14, 2004

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